



Investigating taxon boundaries and extinction risk in endemic Chilean cacti (*Copiapoa* subsection *Cinerei*, Cactaceae) using chloroplast DNA sequences, microsatellite data and 3D mapping

Isabel Larridon^{1,2} , Emily Veltjen², Ilias Semmouri^{2,3}, Pieter Asselman^{2,4}, Pablo C. Guerrero^{5,6}, Milén Duarte^{6,7}, Helmut E. Walter⁸, Mauricio A. Cisternas^{9,10} & Marie-Stéphanie Samain^{2,11}

Summary. *Copiapoa* (Cactaceae) is a genus endemic to the Chilean Atacama Desert. The taxa of *Copiapoa* subsection *Cinerei* occur in an area of high species richness and high levels of species endemism of the Central Chilean biodiversity hotspot. Four taxa are usually recognised in this group: *Copiapoa gigantea* (sometimes placed in *C. cinerea* as *C. cinerea* subsp. *haseltoniana*) and *C. cinerea* including three subspecies (subsp. *cinerea*, subsp. *krainziana* and subsp. *columna-alba*), one of which is often recognised at species level, i.e. the narrow endemic *C. krainziana*. Here, we evaluate the taxon boundaries of *Copiapoa* subsection *Cinerei* using chloroplast sequences and microsatellite data. We generated sequences of three cpDNA markers (*rpl32-trnL*, *trnH-psbA*, *ycf1*) and as indicated in a previous study, found variation between *C. gigantea* and *C. cinerea* on a subsample of 34 individuals. Five microsatellite loci were genotyped for 68 individuals from the known range of *Copiapoa* subsection *Cinerei*. In contrast with expectations, we found relatively high levels of genetic diversity (e.g., $H_e = 0.775 - 0.827$; $H_o = 0.580 - 0.750$) and no population structure, even between the two species. Additionally, species distribution models were conducted based on abiotic suitability and transformed to 3D maps to account for topographical complexity. The species distribution models and their 3D projections support an allopatric distribution of the four taxa of *Copiapoa* subsection *Cinerei*, with each taxon related to a different range with complex topographical features. The obtained molecular results, combined with the presented species distribution modelling, and calculations of extent of occurrence and area of occupancy for the four taxa of *Copiapoa* subsection *Cinerei*, suggest a high extinction risk for most of the taxa. A taxonomic treatment is provided.

Key Words. Atacama Desert, conservation, cpDNA, endemic species, population genetics, species distribution modelling.

Introduction

Copiapoa Britton & Rose is a cactus genus with 32 species endemic to the Central Chilean biodiversity hotspot (Larridon *et al.* 2015). Half of the species as delimited by Hunt *et al.* (2006) were assessed as threatened (IUCN 2015; Larridon *et al.* 2014, 2015). However, Larridon *et al.* (2015) showed that two thirds of *Copiapoa* species need recircumscription and re-evaluation of their conservation status. These authors

provided a taxonomic framework allowing studies below species level in *Copiapoa*. In this study, we focus on patterns of genetic diversity in and between four taxa constituting *Copiapoa* subsection *Cinerei* (Doweld) Helmut Walter & Larridon: *Copiapoa gigantea* Backeb., *C. cinerea* (Phil.) Britton & Rose subsp. *cinerea*, *C. cinerea* subsp. *krainziana* (F. Ritter) Slaba and *C. cinerea* subsp. *columna-alba* (F. Ritter) D. R. Hunt (Fig. 1). These taxa can be found along the Chilean

Accepted for publication 24 September 2018.

¹ Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK. e-mail: i.larridon@kew.org

² Ghent University, Department of Biology, Research Group Spermatophytes, K.L. Ledeganckstraat 35, Gent 9000, Belgium.

³ Ghent University, Faculty of Bioscience Engineering, Laboratory of Environmental Toxicology and Aquatic Ecology, Gent 9000, Belgium.

⁴ Botanic Garden Meise, Nieuwelaan 38, Meise 1860, Belgium.

⁵ Facultad de Ciencias Naturales y Oceanográficas, Departamento de Botánica, Universidad de Concepción, Casilla 160C, Concepción, Chile.

⁶ Instituto de Ecología y Biodiversidad, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

⁷ Universidad de Chile, Departamento de Ciencias Ecológicas, Las Palmeras 3425, Chile.

⁸ The EXSIS Project: cactaceae ex-situ & in-situ conservation, Emmertal 31860, Germany.

⁹ Jardín Botánico Nacional, Camino El Olivar 305, El Salto, Viña del Mar, Chile.

¹⁰ Instituto Multidisciplinario de Biología Vegetal (CONICET-Universidad Nacional de Córdoba), Córdoba, Argentina.

¹¹ Instituto de Ecología, A.C., Centro Regional del Bajío, Avenida Lázaro Cárdenas 253, Pátzcuaro 61600, Michoacán, Mexico.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12225-018-9780-3>) contains supplementary material, which is available to authorized users.

Pacific coast from south of Quebrada Izcuña near Caleta Colorado (24°38'S, 70°33'W) down to the hills north of Chañaral (26°17'S, 70°39'W), an area with high species richness and high levels of species endemism (Guerrero *et al.* 2011a; Walter 2011; Duarte *et al.* 2014), characterised by a hyper-arid precipitation regime and a hyperdesertic bioclimate (Luebert & Pliscoff 2006). The four taxa are largely

allopatrically distributed, but their ranges overlap at the distributional limits (Schulz & Kapitany 1996). South of Taltal (25°24'S, 70°28'W), *C. cinerea* subsp. *cinerea* and *C. cinerea* subsp. *columna-alba* are sympatric, although subsp. *cinerea* only shows a low abundance in this area. Just north of Taltal, the distribution ranges of *C. gigantea*, *C. cinerea* subsp. *cinerea* and *C. cinerea* subsp. *krainziana* partially overlap. However,



Fig. 1. The taxa of *Copiapoa* subsection *Cinerei*: A – *C. gigantea*; D *C. cinerea* subsp. *cinerea*; E *C. cinerea* subsp. *krainziana*; F *C. cinerea* subsp. *columna-alba*. PHOTOS: A, D, E M.-S. SAMAIN; B, C P. C. GUERRERO; F I. LARRIDON.

C. gigantea and *C. cinerea* subsp. *cinerea* are only present there in low abundance. Individuals of presumed hybrid origin have been reported between *C. cinerea* and *C. gigantea*, *C. cinerea* subsp. *cinerea* and subsp. *krainziana*, and *C. cinerea* subsp. *cinerea* and subsp. *columna-alba* (Schulz 2006; Larridon *et al.* 2015).

The four taxa are associated with Mediterranean coastal and inland desert matorral vegetation types (Luebert & Plischoff 2006), with some noticeable differences in ecological preference (Schulz & Kapitany 1996). *Copiapoa gigantea* preferably grows on coastal rocky slopes. In contrast, *C. cinerea* subsp. *cinerea* is found at higher elevations, while subsp. *columna-alba* prefers sandy coastal valleys. The narrow endemic *C. cinerea* subsp. *krainziana* is restricted to hillsides of the San Ramón Valley and its immediate vicinity. No detailed population level studies have been undertaken (Schulz & Kapitany 1996; Schulz 2006; Guerrero *et al.* 2010, 2012), and little information is available concerning ecology, phenology, pollination and seed dispersal in *Copiapoa*. Hoffmann & Walter (2004) state that pollination of *Copiapoa* flowers is performed by insects. According to Hernández-Hernández *et al.* (2014), *Copiapoa* species are mellitophilic (bee-pollinated), with this condition having originated secondarily from species with other pollination syndromes. Schulz (2006) notes that hover flies have been observed as a common pollinator. Seed dispersal is ant-mediated, with the shiny and black seeds having an elaiosome being dispersed by ants over short distances (H. E. Walter, P. C. Guerrero, pers. observ.), and wind may blow seeds released from the fruits some distance from the parent plant as well (Schulz 2006).

Only *Copiapoa cinerea* subsp. *columna-alba* is conserved *in situ*, as there is a population in the Pan de Azúcar National Park (Faundez *et al.* 2013). As for *ex situ* conservation, some living *ex situ* collections of *C. cinerea* and *C. gigantea* are present in Chile at the Jardín Botánico Nacional in Viña del Mar and the Jardín Botánico Aguas Antofagasta. They are also found in living *ex situ* collections outside Chile (Larridon *et al.* 2014). Additionally, the Instituto Nacional de Investigación Agropecuaria (INIA) maintains seeds of *C. cinerea* subsp. *columna-alba* and *C. gigantea* for long term *ex situ* conservation, and seeds are also available in seed banks outside Chile (Larridon *et al.* 2014). *Copiapoa* is included in Appendix II of CITES protecting adult plants from international trade, although only *Copiapoa cinerea* is listed by name (UNEP 2015). However, extraction of plants and seed collection from wild populations continues. A recent global study concluded that almost one-third of cactus species are under threat partly because of over-collection and illegal trade (Goetsch *et al.* 2015).

The main threat to *Copiapoa* taxa in the short term is construction and expansion of coastal roads

(Larridon *et al.* pers. observ., 2013 survey; P. C. Guerrero, pers. observ.). For *C. cinerea* subsp. *krainziana*, gathering of individuals or seeds by cactus collectors is detrimental (Guerrero *et al.* 2010). In the medium and long term, desertification and erosion as a consequence of global climate change may affect populations by diminishing capacity to regenerate (Walter 2011; Guerrero *et al.* 2012). Based on Schulz & Kapitany (1996), Guerrero *et al.* (2010, 2012) estimated that, with the exception of *C. cinerea* subsp. *columna-alba*, overall populations are declining due to generally low numbers of seedling recruitment, high percentage of senile individuals, and declining habitat quality. The conservation status of *C. cinerea* (circumscription including *C. cinerea* subsp. *cinerea*, subsp. *columna-alba* and subsp. *haseltoniana* (Backeb.) N. P. Taylor) according to Hoffmann & Flores (1989), Belmonte *et al.* (1998), and Hoffmann & Walter (2004) is vulnerable (VU); according to Guerrero *et al.* (2012) it is near threatened (NT); and according to Faundez *et al.* (2013) it is of least concern (LC). Saldivia *et al.* (2013) also assessed *C. cinerea* subsp. *krainziana* (as *C. krainziana* F. Ritter) as LC. However, Larridon *et al.* (2014) noted that *C. cinerea* subsp. *krainziana* has high ornamental value for collectors, its narrow distribution range is close to an urbanised area undergoing both touristic and industrial development, and that taxa with small distribution areas are especially vulnerable to environmental changes and global climate change (e.g. Brummitt *et al.* 2015). Previously, it had been assessed as vulnerable by Hoffmann & Flores (1989), Belmonte *et al.* (1998), and Hoffmann & Walter (2004), and even as critically endangered by Guerrero *et al.* (2010). Since species boundaries of *C. cinerea* and *C. gigantea* have been recircumscribed (Larridon *et al.* 2015), their conservation status needs to be reevaluated.

Conserving genetic diversity, preferably *in situ*, is a major objective in conservation management, as it is required for populations to continue to evolve and adapt, and minimise extinction risk (Reed & Frankham 2003; Kramer & Havens 2009; Frankham *et al.* 2010). In the case of the narrow endemic *Copiapoa cinerea* subsp. *krainziana*, conservation is particularly important because it is expected to experience elevated inbreeding, genetic drift and erosion (Ellstrand & Elam 1993; Ouborg *et al.* 2006; Frankham *et al.* 2010), reducing its ability to recover from disturbance and adapt to a changing environment (Hughes *et al.* 2008; Jump *et al.* 2009; Markert *et al.* 2010). Neutral molecular markers can indirectly estimate mating patterns and genetic connectivity, providing valuable information for conservation management (Rossetto & Rymer 2013). Knowledge of extent and structure of genetic diversity helps in choosing conservation units (Funk *et al.* 2012; Dzialuk *et al.* 2014). Few population genetic studies

have been carried out on Cactaceae, often focussing on domesticated species (e.g. Contreras-Negrete *et al.* 2015) instead of on wild populations of threatened or endemic species (Hamrick *et al.* 2002; Figueredo *et al.* 2010; Terry *et al.* 2012; Solórzano *et al.* 2014). Moreover, population genetic studies on cacti of southern South America are comparatively underrepresented in the literature when compared with other regions in the Americas (Tinoco *et al.* 2005; Smith 2013; Contreras-Negrete *et al.* 2015; Bustamante *et al.* 2016). Similarly, globose cacti have been comparatively less examined than columnar cacti (Nassar *et al.* 2003; Tinoco *et al.* 2005; Figueredo *et al.* 2010; Contreras-Negrete *et al.* 2015).

The objectives of this study are to: (1) investigate the taxon boundaries in *Copiapoa* subsection *Cinerei* using chloroplast sequences, microsatellite data, and 3D mapping of their distribution ranges accounting for topographic complexity and based on abiotic suitability; and (2) re-evaluate conservation status by assessing extent of occurrence and area of occupancy. To achieve our objectives, we sequenced three cpDNA markers (*rpl32-trnL*, *trnH-psbA*, *ycf1*) in a subsample of 34 individuals, and genotyped five nuclear microsatellite loci in 68 individuals. As *Copiapoa* subsection *Cinerei* occurs in a landscape fragmented by ridges and valleys, low levels of genetic diversity and some degree of population structure are expected. For neutral genes, uniparental inheritance (e.g. of cpDNA) is expected to reduce effective population size relative to biparentally inherited (nuclear) genes, leading to stronger spatial and temporal differentiation due to genetic drift (e.g. Levy & Neal 1999). Therefore, we expect to find decreased variation and higher population structure in cpDNA data than in the microsatellite dataset.

Materials & Methods

Sampling & Molecular Methods

Total genomic DNA was extracted according to Larridon *et al.* (2015). Three cpDNA markers (*rpl32-trnL*, *trnH-psbA* and *ycf1*) were sequenced based on a previous study of phylogenetic relationships in *Copiapoa* (Larridon *et al.* 2015) in a subsample of 34 individuals (Supplementary Table 1). Samples included in the population genetic study (Table 1) were placed in populations in such a way that each taxon equals a single population to test taxon boundaries. Sampling was carried out throughout the distribution range of *C. gigantea*, *C. cinerea* subsp. *cinerea* and *C. cinerea* subsp. *krainziana* which are largely contiguous, and from the northern population of *C. cinerea* subsp. *columna-alba* as described in Schulz (2006). Although sampling design was aimed at collection of a significant number of individuals from each of the

four studied taxa, difficulties with obtained DNA quantity and quality (a known issue when working with Cactaceae; see e.g. Fehlberg *et al.* 2013) resulted in unequal sampling per population.

Fifteen microsatellite (SSR) marker primer sets published by Terry *et al.* (2006) and Hardesty *et al.* (2008) were tested. Five sets of primers proved polymorphic and were used for analyses: AaB6, AaD9, AaH11 (Terry *et al.* 2006) and mEgR17, mEgR39 (Hardesty *et al.* 2008). Loci were amplified using the Multiplex Master Mix (QIAGEN, Valencia, CA, USA) according to the manufacturer's instruction. PCR products were sized using an ABI3730xl capillary sequencer at MacroGen Europe (Amsterdam, Netherlands), and genotypes scored using Geneious R8 (<http://www.geneious.com>, Kearse *et al.* 2012).

Statistical Methods

ARLEQUIN v. 3.5.1.2. (Excoffier & Lischer 2010) was used to test for Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) across all loci. The allowed missing level per site was set to 0.50. Departure from HWE was determined for each locus per population with 1,000,000 Markov chain steps and 100,000 dememorization steps (Guo & Thompson 1992). LD was determined per population for all pairs of loci using an Expectation Maximization (EM) algorithm with 20,000 permutations and 10 initial codons (Lewontin & Kojima 1960; Slatkin 1994; Slatkin & Excoffier 1996). LD for all pairs of loci across all populations was calculated using FSTAT v. 2.9.3.2. (Goudet 1995, 2002).

GENALEX was used to determine measures of allelic diversity, i.e. polymorphism (P) across all loci, number of alleles (A), number of private alleles (A_p), number of effective alleles (A_e), expected heterozygosity (H_e), and observed heterozygosity (H_o). Testing for HWE per population and F_{IS} (inbreeding coefficient; Weir & Cockerham 1984) was done at 5% nominal p-value and executed in GENEPOP v. 4.3. (Raymond & Rousset 1995; Rousset 2008). Because sample sizes (N) for the populations Pop1 – 4 were unequal, allelic richness (AR) was calculated using a rarefaction method (El Mousadik & Petit 1996) in FSTAT.

In addition to pairwise F_{ST} (fixation index; Weir & Cockerham 1984; Michalakis & Excoffier 1996), we also calculated pairwise R_{ST} (Slatkin 1995), which takes the step-wise mutation model into account, believed to be characteristic of many microsatellites. F_{ST} and R_{ST} values were calculated in ARLEQUIN at 99,999 permutations.

Next, a locus-by-locus AMOVA analysis was performed in ARLEQUIN. The number of different alleles (F_{ST} -like) was used to compute the distance matrix and significance was calculated at 99,999

Table 1. Genetic diversity characteristics per population: sample size (N), percent polymorphic loci (P), number of alleles (A), number of private alleles (A_p), allelic richness (AR), number of effective alleles (A_e), expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{IS}). Standard error provided in parentheses. An asterisk indicates significance from zero at $P < 0.05$. The taxa/populations are listed from north to south.

Population	N	P (%)	A_p	A	AR	A_e	H_e	H_o	F_{IS}
<i>Copiapoia gigantea</i>	18	100	8	8.000 (0.316)	6.076 (0.224)	5.486 (0.268)	0.816 (0.009)	0.567 (0.055)	0.335*
<i>C. cinerea</i>	12	100	5	7.800 (0.800)	6.153 (0.425)	5.465 (0.766)	0.809 (0.026)	0.750 (0.139)	0.124*
subsp. <i>cinerea</i>									
<i>C. cinerea</i>	25	100	8	9.000 (1.225)	5.632 (0.604)	5.157 (1.010)	0.775 (0.041)	0.634 (0.103)	0.216*
subsp. <i>krainziana</i>									
<i>C. cinerea</i> subsp. <i>columna-alba</i>	13	100	5	9.000 (0.894)	6.683 (0.348)	5.960 (0.520)	0.827 (0.016)	0.580 (0.141)	0.341*

permutations. The four taxa were grouped according to the two species: *Copiapoia gigantea* and *C. cinerea*.

Population structure was investigated in STRUCTURE v. 2.3.3. (Pritchard *et al.* 2000). The analysis was run under the admixture model, with 1,000,000 MCMC repetitions after a burn-in of 10,000 and the assumption of correlated allele frequencies. We analysed 10 independent runs wherein K-values were allowed to range from 1 to 15 and no prior information on the population of origin was given. Results were uploaded into STRUCTURE HARVESTER (Earl & von Holdt 2012) to estimate the most likely K (Evanno *et al.* 2005). CLUMPP v. 1.1.2 (Jakobsson & Rosenberg 2007) was used to make a consensus of the results of the independent runs for the optimal K and for K=2 to test the species delimitation, using the Greedy option with random input order and 1,000,000 repeats. Each separate consensus was visualised using DISTRUCT v. 1.1 (Rosenberg 2004).

Genetic diversity between all populations was visualised using DAPC (Discriminant Analysis of Principal Components) ordination in R (R Core Team 2015) with the package “ade4” (Jombart & Ahmed 2011).

Species Distribution Modelling and Assessment of Conservation Status

Predictive distribution modelling was used to infer taxon range extent associated with spatial distribution of environmental suitability. Fifty georeferenced localities of *Copiapoia cinerea* subsp. *cinerea*, 63 of subsp. *columna-alba*, 42 of subsp. *krainziana*, and 127 of *C. gigantea* were used to model the distribution of the taxa. Information on locality data was obtained from different sources: field excursions, literature (Eggli *et al.* 1995; Schulz & Kapitany 1996; Schulz 2006; Guerrero *et al.* 2011a), and Chilean herbaria (CONC, SGO; Thiers continuously updated3). All the compiled occurrences were filtered following two criteria for inclusion: (1) occurrence data based on geographic coordinates or accurate locality information; and (2) occurrences registered after 1950 (revealing present

day distribution). These locality data are not included here, as it concerns CITES listed species under significant threat due to illegal collecting. However, more information may be obtained from the authors. Current climatic variables were obtained from Plissock *et al.* (2014) who modified and corrected biases caused by heterogeneous distribution of data records in northern Chile that were detected for 19 bioclimatic variables of Hijmans *et al.* (2005). Additionally, we used the monthly surface radiation value of the year 2000 (Ohmura *et al.* 1998 and posterior updates), Global Potential Evapo-Transpiration and Global Aridity Index (Zomer *et al.* 2007, 2008). To select variables, a Pearson correlation analysis in ENMTTools (Warren *et al.* 2008) was performed, discarding those variables correlated over 0.9. Twelve variables were retained: Mean Diurnal Range, Isothermality, Max Temperature of Warmest Month, Temperature Annual Range, Mean Temperature of Driest Quarter, Precipitation of Driest Month, Precipitation of Warmest Quarter, Precipitation of Coldest Quarter, Precipitation Seasonality (Coefficient of Variation), Global Potential Evapo-Transpiration and Two Month Radiation (January and October). The resolution of all climatic layers was 1 km². Managing climatic layers was performed with ArcGIS v. 10.1 (ESRI, Redlands, CA, USA).

To model species distributions, species distribution models (SDMs; Elith *et al.* 2011) were generated based on a maximum entropy algorithm implemented in MaxEnt v. 3.3.3 (Phillips *et al.* 2006). The background was selected as the rectangle between -8.98 to 55.97 latitude, and -78.91 to -62.01 longitude. We made 50 replicates (with bootstrap adjustment based on 500 iterations) for each taxon, and used the average models as predicted distributions. The random test percentage of 25% was selected for evaluating the accuracy of each model, and AUC areas were calculated using MaxEnt, which allow evaluating the sensitivity and specificity of the model. Finally, to visualise species distributions accounting for the topographic complexity that characterises the habitat

of *Copiapoa* species we used ArcScene v. 10.1 (ESRI, Redlands, CA, USA).

Quantitative analyses to assess conservation status of the studied taxa based on the SDMs were carried out in ArcMap v. 10.1 (ESRI, Redlands, CA, USA). Specifically, extent of occurrence (EOO) of each taxon was calculated using a convex hull or minimum convex polygon (MCP) on the SDMs (Syfert *et al.* 2014), and area of occupancy (AOO) was calculated by the sum of the area where the species is distributed according to SDMs. Based on these calculations of the EOO and AOO, a preliminary conservation status of taxa was assessed in line with IUCN Red List criteria (IUCN 2012). The EOO and AOO were also calculated in GeoCAT (Bachman *et al.* 2011) based on point data, where the AOO was based on a user defined cell width of 2 km in line with IUCN Red List criteria (IUCN 2012).

Results

Plastid DNA Markers

Although the markers *rpl32-trnL*, *trnH-psbA* and *ycf1* used here were previously identified as useful for reconstructing molecular phylogenetic relationships in the genus *Copiapoa* (Larridon *et al.* 2015), sequencing results of the cpDNA markers obtained in this study revealed no variation within taxa, and little among taxa. *Copiapoa cinerea* consistently varies from *C. gigantea* by one nucleotide insertion in *rpl32-trnL*, one nucleotide insertion in *trnH-psbA*, and three nucleotide substitutions in *ycf1*. *Copiapoa cinerea* subsp. *krainziana* consistently varies from all other *Copiapoa* subsection *Cinerei* taxa by one nucleotide insertion and one nucleotide substitution in *rpl32-trnL*. The obtained sequences of each plastid marker were submitted to GenBank (Supplementary Table 1).

SSR Loci

Results showed no evidence of LD between pairwise loci. Out of ten locus pairs, zero pairs showed consistent LD patterns. Only AaH11 × mEgR39 and AaB6 × mEgR17 in population Pop3, and AaB6 × mEgR39 in population Pop4 show significant lower P-values than the adjusted 0.005 (Bonferroni correction) (Supplementary data Table 2). Significant deviations from HWE ($P < 0.05$) were found for loci AaD9 and mEgR17 (Supplementary data Table 3).

Genetic Diversity & Intra-population Characteristics

All loci were polymorphic (P value of 100%) for all populations (Table 1). The four taxa show high values of observed heterozygosity (H_o), and higher values of expected heterozygosity (H_e) (Table 1). H_o and H_e deviate significantly as F_{IS} was significantly greater than zero (Table 1) and the populations show comparable allelic diversity (A_p , A , AR and A_e ; Table 1).

Genetic Structure & Gene Flow between Populations

Pairwise F_{ST} and R_{ST} values of the taxa are low to moderate (Wright 1978; Hartl & Clark 1997) (Table 2). Only the pairwise R_{ST} value for *Copiapoa gigantea*–*C. cinerea* subsp. *cinerea* is not significantly different from zero. Looking at the R_{ST} values (Table 2), *C. cinerea* subsp. *columna-alba* appears the most differentiated taxon. However, the F_{ST} values are within the same range (Table 2).

The results of the locus-by-locus AMOVA analysis on the dataset (Supplementary data Table 4) show that 4.51% of the variation in the data is significantly explained by respecting the circumscription of the two species (F_{CT} : 0.045*). Further dividing the species *Copiapoa cinerea* into its three subspecies significantly explains another 3.39% of the variation (F_{SC} = 0.036*). The F_{ST} value of 0.079, representing the variance explained by respecting all four taxa compared to the total variance, is also significantly different from zero. However, >92% of the variation remains allocated to individual differences within the taxa.

The L(K) plot shows that at K=1 the highest mean value was found, which slightly decreases to K=2 and K=3, then strongly decreasing for all further Ks (Supplementary Fig. 1). Moreover, the SD values are increasing being lowest at K=1, higher at K=2 and even higher at K=3 (Supplementary Fig. 1). Therefore, the observed “peak” of DeltaK at K=3 and K=4 (Supplementary Fig. 1), is likely an artefact of the increasing SD values and slight negative changes of L(K), due to low genetic differentiation. This is confirmed by the pattern at K=4 (Fig. 2A), which clearly shows that there is no structure: all 4 “gene pools” are present in nearly equal proportion in each individual in each of the four taxa. The STRUCTURE analysis does not give any indication of population structure. Rather it clearly indicates that the four taxa are only very weakly genetically differentiated. Together this results in K=1 being the most parsimonious number of gene pools. However, STRUCTURE results for K=2 (Fig. 2B) at least indicates some difference of genepool frequency between the two species *Copiapoa gigantea* and *C. cinerea*. However, there is no statistical support for K=2.

DAPC analysis of the dataset result in the first (horizontal) axis, which explains most of the variation in the dataset, splitting the taxa in two main groups along species boundaries (Fig. 3).

Distribution, EOO & AOO

The species distribution modelling in a 3D framework supports the observed allopatric distribution among taxa, although they can overlap to some extent along boundaries (Fig. 4). For *Copiapoa gigantea*, our analysis shows that this species is mainly distributed in coastal habitats north of Taltal (Fig. 4A), while *C. cinerea* subsp. *cinerea* is distributed along the coast and somewhat inland near Taltal (Fig. 4B). In the case of *C. cinerea* subsp. *krainziana*, distribution is presented by

Table 2. Pairwise estimates of fixation (F_{ST} , R_{ST}) between the taxa/populations. An asterisk indicates significance from zero.

F_{ST}	<i>C. gigantea</i>	subsp. <i>cinerea</i>	subsp. <i>krainziana</i>	subsp. <i>columna-alba</i>	R_{ST}	<i>C. gigantea</i>	subsp. <i>cinerea</i>	subsp. <i>krainziana</i>	subsp. <i>columna-alba</i>
<i>Copiapoa gigantea</i>									
<i>C. cinerea</i>	0.051*				-0.038				
subsp. <i>cinerea</i>									
<i>C. cinerea</i> subsp. <i>krainziana</i>	0.065*	0.036*			0.096*	0.067*			
<i>C. cinerea</i> subsp. <i>columna-alba</i>	0.049*	0.027*	0.096*		0.125*	0.137*	0.116*		

a single point since georeferenced localities for this narrow endemic occur so close together that distribution could not be modelled at this scale (Fig. 4C). The taxon with the southernmost distribution is *C. cinerea* subsp. *columna-alba* (Fig. 4D). Interestingly, this taxon has expanded its range covering both coastal and more inland localities. *Copiapoa gigantea* and *C. cinerea* subsp. *columna-alba* are the most widely distributed taxa of *Copiapoa* subsection *Cinerei*. Calculated EOO and AOO values of *C. gigantea* and *C. cinerea* (including all subspecies) suggest a preliminary conservation status assessments of EN for all taxa except *C. cinerea* subsp. *krainziana*, which is assessed as CR (Table 3).

Discussion

Plastid DNA Markers

Although little variation in sequence data was observed, the observed variation supports the species

boundaries of *Copiapoa gigantea* and *C. cinerea*. Below the species level, only *C. cinerea* subsp. *krainziana* shows some sequence variation from the other two subspecies. The amount of sequence variation observed between the taxa included in this study is comparable for other groups of *Copiapoa* species that have distribution ranges which occur in close proximity to each other, such as the taxa of *Copiapoa* section *Echinopoa* or *C.* section *Mammillopoa* (Larridon *et al.* 2015). In general, limited genetic diversity in Cactaceae was also observed in other studies using chloroplast sequence data (e.g. Majure *et al.* 2012).

SSR Loci

As no consistent, supported evidence of LD between pairwise loci was found (Supplementary data Table 2), we assume no linkage between markers. Significant deviation from HWE in loci can indicate null alleles, genotyping errors, recent admixture, or unrepresentative (nonrandom) sampling. Here, deviations were observed in loci AaD9 and mEgR17 (Supplementary data Table 3). Assuming deviations are due to underrepresentation of true population genetic diversity, and because analyses ran excluding loci AaD9 and mEgR17 yielded similar results only with less predictive power, all five loci were kept for the final analyses. For future studies it is advisable to develop additional SSR markers *de novo* using next-generation sequencing, as recently proved successful for *Echinopsis chiloensis* (Ossa *et al.* 2016), to (1) increase statistical power and (2) because the successful cross-amplification of the markers used in this study indicates that ancient loci were targeted.

Intra-population Genetic Structuring

Allelic diversity may be similar in the four taxa because of occasional historical gene flow between them (resulting in hybrids), or as a result of their recent speciation (Larridon *et al.* 2015). Allelic diversity (AR , A_e) of *Copiapoa cinerea* subsp. *krainziana* is slightly lower compared to the other taxa, possibly due to small sampling range (sampled individuals grew close to-

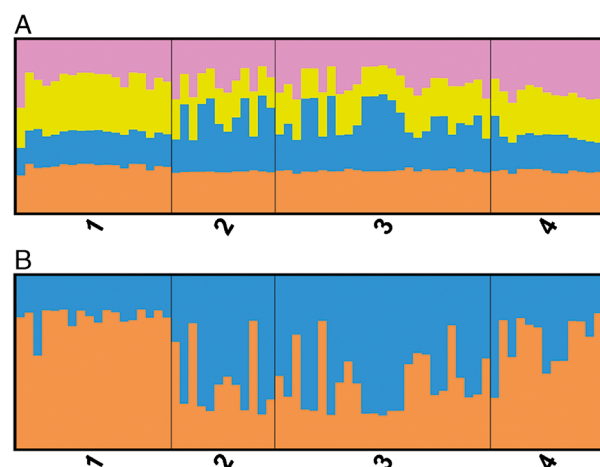


Fig. 2. Genetic structure of the taxa: 1 *Copiapoa gigantea*; 2 *C. cinerea* subsp. *cinerea*; 3 *C. cinerea* subsp. *krainziana*; 4 *C. cinerea* subsp. *columna-alba* inferred by Bayesian cluster analysis using allelic data from 5 microsatellite loci. A STRUCTURE result for K=4; and B STRUCTURE result for K=2.

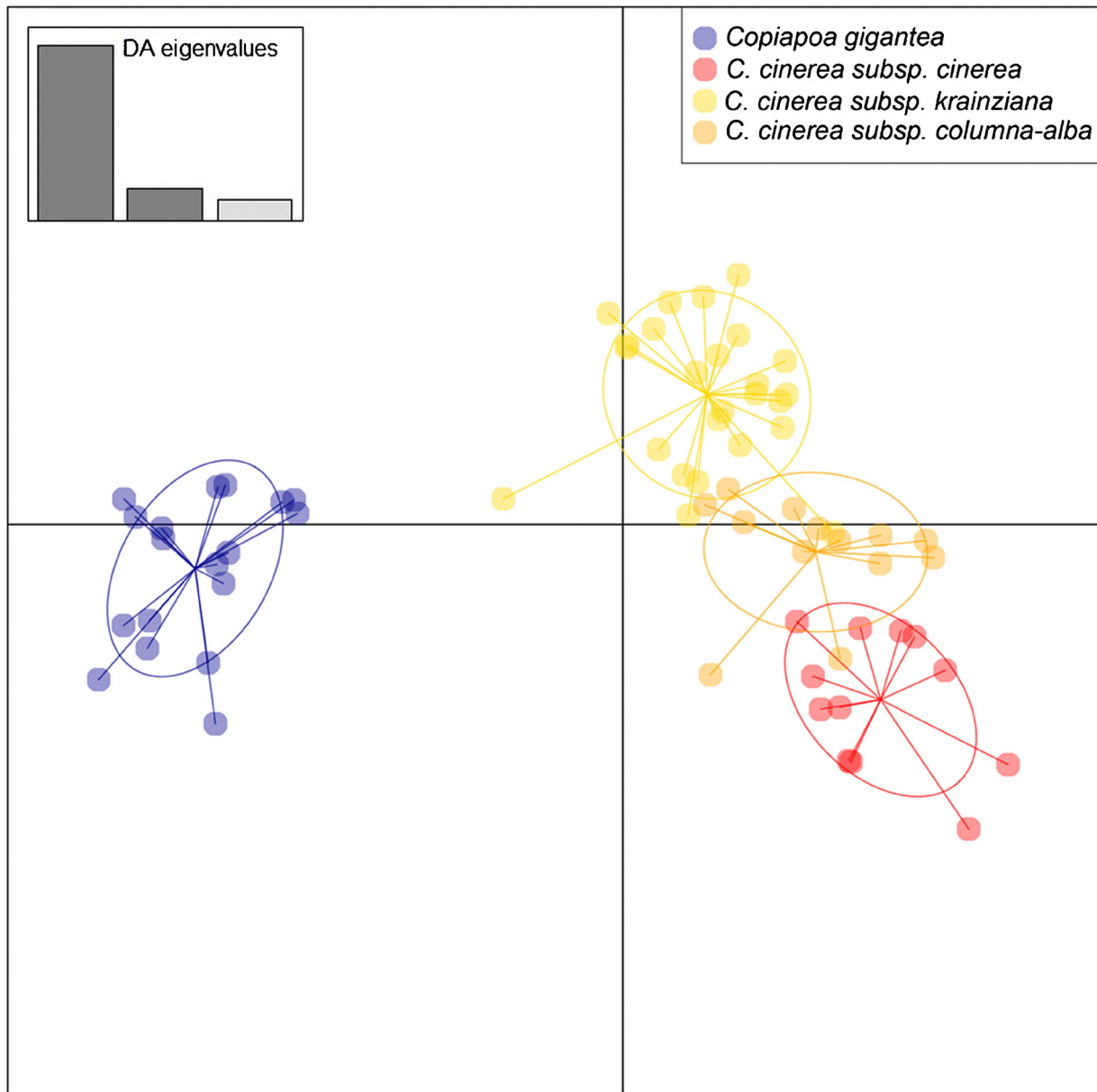


Fig. 3. Results of DAPC showing *Copiapoa gigantea* (blue); *C. cinerea* subsp. *cinerea* (red); *C. cinerea* subsp. *krainziana* (yellow); and *C. cinerea* subsp. *columna-alba* (orange). PCA's retained = 37. Number of discriminant functions retained: 3.

gether, whilst individuals sampled of other taxa occurred over a larger range). Nonetheless, sampling reflects the natural state since *C. cinerea* subsp. *krainziana* is a narrow endemic (Fig. 4).

Compared with a population genetic study of *Astrophytum asterias* using some of the same microsatellite markers and similar sample size per population (Terry *et al.* 2012), a higher number of alleles per population was retrieved in this study (Table 1). Comparing values in Table 1 with those obtained for wild populations of *Mammillaria huitzilopochtli* and *M. supertexta* (Solórzano *et al.* 2014), they are of a similar level. F_{IS} was significantly greater than zero (Table 1) in all populations, suggesting a level of inbreeding within each of the four studied taxa.

This detected inbreeding could be due to: (1) a low number of individuals per taxon leading to a higher level of homozygosity; or (2) reproductive biology.

Genetic Structure & Gene Flow between Populations

Pairwise F_{ST} values between the populations are significant, so as to not support random mating, but low, indicating (past) gene flow among the different taxa studied (Table 2). Although slightly higher F_{ST} values were recorded, we expected *Copiapoa gigantea* to have a higher degree of genetic differentiation (and hence fixation) compared to the subspecies of *C. cinerea*, because genetic differentiation was detected in the

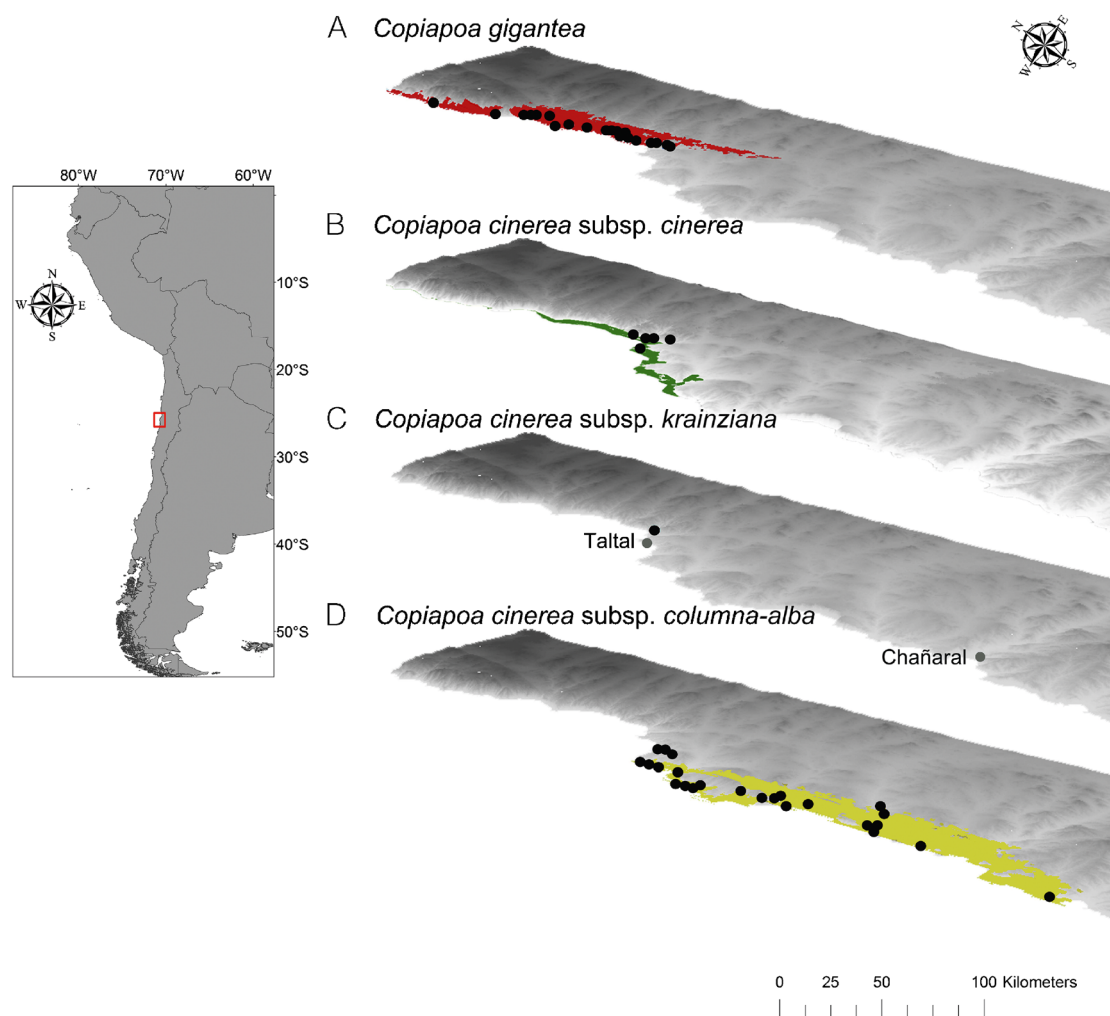


Fig 4. Map showing the modelled distributions of the four taxa of *Copiapoa* subsection *Cinerei*, and their actual occurrences. **A** *C. gigantea* (red: modelled distribution; black: georeferenced observations); **B** *C. cinerea* subsp. *cinerea* (green: modelled distribution; black: georeferenced observations); **C** *C. cinerea* subsp. *krainziana* (black: georeferenced observations); and **D** *C. cinerea* subsp. *columna-alba* (yellow: modelled distribution; black: georeferenced observations).

cpDNA sequence data (this study; Larridon *et al.* 2015). The lower than expected fixation may be due to hybridisation events that result in gene flow and limit fixation. The latter is supported by reports of hybrids between *C. gigantea* and *C. cinerea* (e.g. Larridon *et al.* 2015). Another reason for the lack of concordance between the differentiation estimates based on chloroplast markers (Larridon *et al.* 2015) and the nuclear markers used in this study may be explained by deviations in the levels of gene dispersal mediated by pollen and seeds. The seeds may be dispersed more locally (i.e. ant-mediated and/or wind-mediated seed dispersal, see above), a pattern reflected in the genetic differentiation observed when using maternally inherited chloroplast markers (Larridon *et al.* 2015). In contrast, pollen might be travelling over longer distances due to pollinator's behaviour (i.e. bees and/or hover flies, see above), and hybridisations might occur resulting in estimates based on nuclear markers (bipa-

rentally inherited) showing less structure. More research is needed on the ecology of these species and their interaction with their pollinators and seed dispersers.

Based on the R_{ST} values (Table 2), *Copiapoa cinerea* subsp. *columna-alba* is noted as the most differentiated taxon, instead of *C. gigantea*. However, the higher fixation expressed in pairwise R_{ST} values is countered by the fact that South of Taltal, *C. cinerea* subsp. *cinerea* and subsp. *columna-alba* are sympatric, and occasional hybrids have been reported (Schulz 2006).

Dividing the data in two species, *Copiapoa gigantea* and *C. cinerea*, explains the variance (F_{CT}) significantly better compared to the total variance, as does splitting the data into the four separate taxa (F_{ST}) (Supplementary Table 4). Dividing the dataset first into two species and then into four taxa is also significant (F_{SC}). However, 92% of variation remains explained by differences within taxa (Supplementary Table 4). We cannot exclude that limited sampling influenced these results.

Table 3. EOO and AOO of the four taxa of *Copiapoa* subsection *Cinerei* calculated as Species Distribution Models (SDM) in ArcGIS and generated based on point data in GeoCAT with mention of the preliminary assessment of their conservation status suggested by these values according to IUCN criteria (IUCN 2012).

Taxon	EOO (km ²)		AOO (km ²)		Assessment	
	Model based EOO	Point based EOO	Model based AOO	Point based AOO	Model based rating	Point based rating
<i>C. gigantea</i>	901.8	633	612.5	124	EN	EN
<i>C. cinerea</i> subsp. <i>cinerea</i>	861.3	57	570.2	24	EN	CR–EN
<i>C. cinerea</i> subsp. <i>krainziana</i>	-	8	-	8	CR	CR
<i>C. cinerea</i> subsp. <i>columna-alba</i>	1230.3	683	1002.3	56	EN	EN
<i>C. cinerea</i> (all subspecies)	2993.4	1260	2185.0	80	EN	EN

Note. GeoCAT generates the EOO value in GeoCAT using a convex hull or minimum convex polygon (MCP). If two populations are separated by clearly unsuitable areas, e.g. sea, then this will be included in the area measurement. Following Gaston & Fuller (2009), the EOO is measured including these discontinuities, thereby retaining a clear distinction between EOO and AOO (<http://geocat.kew.org/>).

Although genetic structure of the studied loci does not follow our predefined species (*Copiapoa gigantea* and *C. cinerea*) or populations (four taxa) as expected, we find that in the STRUCTURE results for K=2 (Fig. 2B) individuals of *C. gigantea* mainly tend to belong to one genetic unit while most individuals of *C. cinerea* subsp. *cinerea* and subsp. *krainziana* belong to the other genetic unit. *Copiapoa cinerea* subsp. *columna-alba*, the most widely distributed *C. cinerea* subspecies, is not as clearly defined, possibly due to undersampling and/or the inability of the markers to distinguish it. It is likely that structure analysis results will improve with a larger sampling of individuals and markers since the taxa may have more private alleles that were not sampled in this study. Another explanation for the lack of genetic structure is a high level of (past) gene flow between the populations of the four taxa. This is in contrast with structure analysis results found for *Coryphantha robustispina* (Schott ex Engelm.) Britton & Rose, where based on microsatellite loci three subspecies proved genetically distinct (Baker & Butterworth 2013).

DAPC results (Fig. 3) split the four taxa into two main groups supporting the species boundaries between *Copiapoa gigantea* and *C. cinerea*.

When we align DAPC results (Fig. 3) with pairwise F_{ST} and R_{ST} values (Table 2), the results support each other in that we have, on the one hand, the genetic divergence of *Copiapoa gigantea* from the *C. cinerea* subspecies complex (F_{ST}), and on the other hand *C. cinerea* subsp. *columna-alba* appearing more differentiated from the two other *C. cinerea* subspecies (R_{ST}). The latter indicates once more that *C. cinerea* subsp. *columna-alba* very likely houses more genetic variation than was sampled here. This is very likely since southerly populations of this subspecies could not be sampled since they occur in the Pan de Azúcar National Park.

Distribution & Taxonomic Divergence

Summarising the cpDNA and SSR results, it appears that species boundaries in *Copiapoa* subsection *Cinerei* receive some support in terms of sequence variation in cpDNA markers and some statistical analyses of the SSR data (e.g. DAPC; Fig. 3), but no statistically supported genetic structure is found (Fig. 2; Supplementary Fig. 1). Genetic differentiation between the subspecies of *C. cinerea* receive even less support based on the molecular data studied. However, the taxa are clearly distinct morphologically (Fig. 1, Key to species of subsection *Cinerei*), and for the most part allopatrically distributed although their ranges overlap at their distributional limits (Fig. 4). All studied taxa occupy distinct areas among the high topographic complexity along the coastal range of the Chilean Atacama Desert. For example, *C. gigantea* is mainly distributed along the coast north of Taltal, while *C. cinerea* subsp. *krainziana* is only found in the hills and valleys northeast of Taltal, and *C. cinerea* subsp. *columna-alba* is distributed in the coastal range south of Taltal. Phylogenetic analyses of the genus revealed that although many *Copiapoa* species occur sympatrically, sister taxa are segregated in geographic space (Larridon *et al.* 2015). Similarly to other Chilean cacti (i.e. *Eriosyce* Phil. subg. *Neoporteria* Helmut Walter), taxonomic divergence within *Copiapoa* subsection *Cinerei* may have occurred through isolation by distance favoured by the high topographic complexity in the coastal zone of the Atacama Desert (Guerrero *et al.* 2011b), together with historic climatic changes that modified vegetation belts and the distribution of species within a hyperarid landscape (Gayo *et al.* 2012). Overlap at distributional limits may likewise be part of the mechanism of diversification since new taxa could have originated or are still evolving from hybridisation events. Two hypotheses could be tested in future studies: (1) speciation through spatial isolation and posterior

secondary contact, and (2) speciation through hybridisation and posterior migration.

Relevance for Conservation

Correct species delimitation matters for conservation because we need to know what the units for conservation are, generally at the species level, to optimally invest resources in the species or area of interest. According to the IUCN Red List, the conservation status of *Copiapoa cinerea* (circumscription including *C. cinerea* subsp. *cinerea*, subsp. *columna-alba* and subsp. *haseltoniana*) is LC (Faundez *et al.* 2013). Our analyses suggest that the former assessment might underestimate the real extinction risk of *C. cinerea* and *C. gigantea*, since the species delimitation used species lumped into an artificial taxon, increasing the geographic range on which the assessment was based. Our results provide support for the species boundaries of *C. cinerea* and *C. gigantea* as recircumscribed by Larridon *et al.* (2015), with *C. cinerea* including subsp. *cinerea*, subsp. *krainziana* and subsp. *columna-alba*. This highlights the need to advance taxonomic re-evaluation of genera and species with unclear evolutionary relationships and or delimitation, where species numbers might be inflated (Isaac *et al.* 2004) or underestimated. We have updated the conservation assessments in the Taxonomic Treatment below. Assessing levels of hybridisation, and testing whether hybrids have equal fitness compared to their parental populations can provide further insights. Implementation of conservation measures such as increased control of illegal

harvesting and habitat conservation management (e.g. in the form of protected areas) are here proposed (see also Walter 2011). For *Copiapoa* species in general, there is an urgent need for more ecological data, more specifically concerning the reproductive interaction of the taxa with their pollinators and seed dispersers to make inferences on potential pollen and seed dispersal-mediated gene flow and its influence on taxon limits.

Taxonomic Treatment

Members of *Copiapoa* subsection *Cinerei* are mound-forming or few to single stemmed, the stem tissue is very hard and the stems are \pm pruinose, with up to 40 ribs that are usually less than 1.2 cm high, and their roots are always fascicular. The two species can easily be distinguished from each other morphologically, because the stems of *C. cinerea* individuals form loose groups or are solitary, have grey apical wool, and diameters less than 20 cm, while *C. gigantea* individuals form large dense mounds, have (orange)-brown apical wool, and stem diameters up to 25 cm. The three subspecies of *C. cinerea* can also be differentiated from each other based on their morphology (Key to species of subsection *Cinerei*; Fig. 1). Descriptions, identification keys to the species and subspecies, and nomenclature have been adapted from H. E. Walter, *Flora de Chile*, Cactaceae, Vol. 24 (in preparation), and follow the classification suggested by Larridon *et al.* (2015) and the results presented here. Only the most commonly used synonyms are provided.

Key to species of subsection *Cinerei*

1. Plants forming large dense mounds; apical wool (orange)-brown 1. *C. gigantea*
Plants forming loose groups or solitary; apical wool grey. 2. *C. cinerea*
2. Stems generally branching basically and/or laterally; ribs up to 26, <2 cm broad, not undulating 3
Stems solitary, very rarely branching laterally; ribs up to 40, <1 cm broad, undulating. 2c. *C. cinerea* subsp. *columna-alba*
3. Stems somewhat pruinose; hair-like spines up to 30 per areole, completely hiding superior part of stem, flexible, some contorted, mostly whitish 2b. *C. cinerea* subsp. *krainziana*
Stems heavily pruinose; spine number <10, subulate, straight, generally black and thick. . . 2a. *C. cinerea* subsp. *cinerea*

1. *Copiapoa gigantea* Backeb. (Backeberg 1936: 104)
Copiapoa haseltoniana Backeb. (Backeberg 1957: 33);
Copiapoa cinerea subsp. *haseltoniana* (Backeb.) N. P. Taylor (1997: 8).
Copiapoa eremophila F. Ritter (1980: 1104 – 1105).
Copiapoa tenebrosa F. Ritter (1980: 1098 – 1099).

ILLUSTRATION. Hoffmann & Walter (2004: 134 – 135); Fig. 1A – C.

Plants branching basally and laterally, forming huge mounds up to 2 m diam. and 1 m high; stems elongated cylindrical, up to 25 cm diam., epidermis

pale grey-green to grey, \pm pruinose; apical wool orange-brown. *Roots* fascicular. *Ribs* broad, up to c. 40, tuberculate, notched below areoles. *Areoles* large, c. 1 cm, round-oval, up to 2 cm apart. *Spines* all nearly equally long, 2 – 4 cm, thickly acicular, mostly straight, honey-coloured; radials c. 3 – 7, porrect; centrals 0 – 1. *Flowers* broad funnel-form, pale yellow, 4 – 4.5 cm; bract scales reddish brown, axils naked; external perianth segments often red-tipped; pericarpel somewhat constricted above; nectary broad; ovary compressed. *Fruits* pale red, oval, 1.5 cm, some scales near rim. *Seeds* oval to pyriform, c. 1.5 mm; hilum oval, position nearly lateral.

DISTRIBUTION & HABITAT. *Copiapoa gigantea* preferably grows on coastal rocky slopes along the Chilean Pacific coast from south of Quebrada Izcuña near Caleta Colorado (24°38'S, 70°33'W) to north of Taltal (25°24'S, 70°28'W) (Fig. 4A).

CONSERVATION STATUS. In the taxonomic framework used for the IUCN Red List, this taxon was considered under its synonym *Copiapoa cinerea* subsp. *haseltoniana*. Faundez *et al.* (2013), assessed *C. cinerea* (circumscription including *C. cinerea* subsp. *cinerea*, subsp. *columna-alba* and subsp. *haseltoniana*) as LC. Although Hoffmann & Flores (1989) and Hoffmann & Walter (2004) considered this taxon on its own to be VU, the conservation status of *C. gigantea* has not been assessed using the IUCN criteria (IUCN 2012). The EOO and AOO values (Table 3) indicate that *C. gigantea* may be considered as EN if conforming to additional conditions listed in Criterion B (IUCN 2012). The species is mainly threatened by construction and expansion of roads (I. Larridon, pers. observ.; P. C. Guerrero, pers. observ.), resulting in a decline of the extent and/or quality of habitat. Although it has large number of individuals, Schulz & Kapitany (1996) & Guerrero *et al.* (2010, 2012) estimated that overall population trends are negative. The species is currently not protected *in situ*, although it is conserved in *ex situ* seed bank and living collections (Larridon *et al.* 2014).

2. *Copiapoa cinerea* (Phil.) Britton & Rose (1922: 86); *Echinocactus cinereus* Phil. (Philippi 1860: 23).

ILLUSTRATION. Hoffmann & Walter (2004: 130 – 133, 152 – 153); Fig. 1D – F.

Plants generally branching laterally and/or basally, sometimes forming loose mounds; stems globose to elongated-cylindrical, up to c. 100 × 18 cm; hard, grey-green, ± pruinose; apical wool white or grey. *Roots* fibrous. *Ribs* obtuse, 12 – 26, up to 2 cm broad and up to 1.5 cm high, hardly widened at areoles, slightly tuberculate. *Areoles* round, <7 mm, grey to black, 1 – 1.5 cm apart. *Spines* variable, few (<10), usually subulate, generally black, turning grey, straight rarely slightly bent, mostly <3 cm; radials 0 – 6, usually <2 cm; centrals 0 – 4, thicker and somewhat longer. *Flowers* funnel-form, pale yellow, c. 2.5 – 3.5 cm; interior perianth segments often tipped red, external ones with reddish mid-stripe; a few small pink bract scales only on rim of pericarpel and lower portion of hypanthium; axils naked; pericarpel offset from hypanthium; ovary compressed; nectary large. *Fruit* pinkish-red, 1 – 1.5 cm, with c. 5 small, red bract scales. *Seeds* ovoid to c. 1.5 mm; testa smooth; hilum large, narrow oval, position oblique.

DISTRIBUTION & HABITAT. See subspecies (Fig. 4B – D).

CONSERVATION STATUS. According to the IUCN Red List, the conservation status of *Copiapoa cinerea* (circumscrip-

tion including *C. cinerea* subsp. *cinerea*, subsp. *columna-alba* and subsp. *haseltoniana*) is LC (Faundez *et al.* 2013). Using the species delimitation as accepted by Larridon *et al.* (2015) and this study, even the total EOO and AOO values (Table 3) for *C. cinerea*, indicate that it may be considered as EN if conforming to additional conditions listed in Criterion B (IUCN 2012). Although Schulz & Kapitany (1996) and Guerrero *et al.* (2010, 2012) estimated that, overall population trends are negative due to generally low numbers of seedling recruitment, high percentage of senile individuals, and declining habitat quality, this negative trend is not followed by *C. cinerea* subsp. *columna-alba*. As a species therefore, the conservation status can be maintained as LC.

2a. *Copiapoa cinerea* (Phil.) Britton & Rose subsp. *cinerea*

Copiapoa cinerea var. *albispina* F. Ritter (1963: 30).

ILLUSTRATION. Hoffmann & Walter (2004: 130 – 131); Fig. 1D.

Plants branching laterally and basically, sometimes forming clumps. *Ribs* ≤ 26, broad and deep, scarcely undulating. *Perianth* segments tipped red, external ones with red mid-stripe. *Seeds* to 1.5 mm.

DISTRIBUTION & HABITAT. *Copiapoa cinerea* subsp. *cinerea* is found at higher elevations in the region of the Chilean coastal town of Taltal (25°24'S, 70°28'W) (Fig. 4B).

CONSERVATION STATUS. Faundez *et al.* (2013) stated that if *Copiapoa cinerea* subsp. *cinerea* were considered as a separate species it would qualify as VU B1a(iii,v). Our calculated EOO and AOO values (Table 3) suggest that the subspecies should be considered as EN instead. The subspecies is currently not protected *in situ*, although it is conserved in *ex situ* seed bank and living collections (Larridon *et al.* 2014).

2b. *Copiapoa cinerea* subsp. *krainziana* (F. Ritter) Slaba (1997: 3); *Copiapoa krainziana* F. Ritter (1963: 30).

Copiapoa krainziana var. *scopulina* F. Ritter (1963: 30).

ILLUSTRATION. Hoffmann & Walter (2004: 152 – 153); Fig. 1E.

Plants branching, forming loose mounds; stems grey-green often slightly pruinose, to 20 cm diam. *Ribs* not undulating. *Spines* thin and numerous, completely hiding superior part of stem, variable in colour and attitude, white to greyish black, porrect to radiating, straight to curved or contorted, finely acicular, flexible, to 3.5 cm.

DISTRIBUTION & HABITAT. The narrow endemic *Copiapoa cinerea* subsp. *krainziana* is restricted to hillsides of the

San Ramón Valley and its immediate vicinity located just north of Taltal (25°24'S, 70°28'W) (Fig. 4C).

CONSERVATION STATUS. Saldivia *et al.* (2013) listed the conservation status of *Copiapoa cinerea* subsp. *krainziana* (as *C. krainziana*) as LC. According to our results the conservation status of this taxon can be assessed at least as VU based on its very small AOO (AOO < 20 km²). Besides its very small EOO and AOO (Table 3), the fact that it is only known from two or three adjacent populations that can be considered as a single location, impacts on habitat quality due to drought as a result of global climate change, as well as threats due to illegal plant collecting on the number of mature individuals, it can be assessed as CR (IUCN 2012). Conservation action for this taxon is particularly important because our results suggest elevated inbreeding, and as a narrow endemic it may also experience genetic drift and erosion. *Copiapoa cinerea* subsp. *krainziana* should also be monitored closely as ongoing speciation may be limited due to hybridisation with individuals of *C. cinerea* subsp. *cinerea*. The subspecies is currently not protected *in situ*, although it is conserved in *ex situ* seed bank and living collections (Larridon *et al.* 2014).

2c. *Copiapoa cinerea* subsp. *columna-alba* (F. Ritter) D. R. Hunt (2002: 13); *Copiapoa columna-alba* F. Ritter (1959: 199 – 200).

Copiapoa columna-alba var. *nuda* F. Ritter (1980: 1095).
Copiapoa melanohystrix F. Ritter (1980: 1096).

ILLUSTRATION. Hoffmann & Walter (2004: 132 – 133); Fig. 1F.

Plants solitary, rarely branching laterally; ribs to c. 40 (– 50), up to 1 cm broad, flatter, undulating. *Perianth* segments not tipped red, nor with mid-stripes. *Seeds* smaller.

DISTRIBUTION & HABITAT. *Copiapoa* subsp. *columna-alba* prefers sandy coastal valleys along the Chilean Pacific coast from south Taltal (25°24'S, 70°28'W) down to the hills north of Chañaral (26°17'S, 70°39'W) (Fig. 4D).

CONSERVATION STATUS. The EOO and AOO values (Table 3) indicate that *Copiapoa cinerea* subsp. *columna-alba* may be considered as EN if conforming to additional conditions listed in Criterion B (IUCN 2012). However, since this is the subspecies with the largest number of individuals and distribution range, a negative population trend has not been estimated (Schulz & Kapitany 1996; Guerrero *et al.* 2010, 2012), it occurs in the National Park Pan de Azúcar, and is also conserved in *ex situ* seed bank and living collections (Larridon *et al.* 2014), its conservation status can be assessed as LC.

Acknowledgements

The research by I.L. was supported by the Special Research Fund (postdoctoral fellowship grant n° B/13089/19, Ghent University) and the research by I.L., E.V., I.S., P.A., and M.-S.S. by Fondation Franklinia (grant n° E/01394/01, Ghent University). Research by M.-S.S. was also supported by the Instituto de Ecología, A.C., project n° 11337. P.C.G. research was supported by FONDECYT grant n° 1160583. The herbaria CONC and SGO are thanked for providing occurrence data. Fieldwork was funded by grants from Research Foundation Flanders and King Leopold III Foundation for the Exploration and Protection of Nature, with support of the Jardín Botánico Nacional of Chile.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Bachman, S., Moat, J., Hill, A. W., de la Torre, J. & Scott, B. (2011). Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. In: V. Smith & L. Penev (eds), e-Infrastructures for data publishing in biodiversity science. *ZooKeys* 150: 117 – 126. <http://geocat.kew.org/> (accessed 20.11.2015).
- Backeberg, C. (1936). *Copiapoa gigantea*. *Jahrb. Deutsch. Kakteen-Ges.* 1: 104.
- (1957). *Copiapoa haseltoniana*. *Descriptiones Cactearum Novarum*: 33. G. Fischer, Jena.
- Baker, M. C. & Butterworth C. A. (2013). Geographic distribution and taxonomic circumscription of populations within *Coryphantha* section *Robustispina* (Cactaceae). *Amer. J. Bot.* 100: 984 – 997.
- Belmonte, E., Faúndez, L., Flores, J., Hoffmann, A., Muñoz, M. & Teillier, S. (1998). Categorías de conservación de las cactáceas nativas de Chile. *Bol. Mus. Nac. Hist. Nat. Santiago de Chile* 47: 69 – 89.
- Britton, N. L. & Rose, J. N. (1922). *The Cactaceae*, Vol. 3. Carnegie Institute, Washington D.C.
- Brummitt, N. A., Bachman, S. P., Griffiths-Lee, J., Lutz, M., Moat, J. F., Farjon, A., Donaldson, J. S., Hilton-Taylor, C., Meagher, T. R., Albuquerque, S., Aletrari, E., Andrews, A. K., Atchison, G., Baloch, E., Barlozzini, B., Brunazzi, A., Carretero, J., Celesti, M., Chadburn, H., Cianfoni, E., Cockel, C., Coldwell, V., Concetti, B., Contu, S., Crook, V., Dyson, P., Gardiner, L., Ghanim, N., Greene, H.,

- Groom, A., Harker, R., Hopkins, D., Khela, S., Lakeman-Fraser, P., Lindon, H., Lockwood, H., Loftus, C., Lombrici, D., Lopez-Poveda, L., Lyon, J., Malcolm-Tompkins, P., McGregor, K., Moreno, L., Murray, L., Nazar, K., Power, E., Quiton Tuijelaars, M., Salter, R., Segrott, R., Thacker, H., Thomas, L. J., Tingvoll, S., Watkinson, G., Wojtaszekova, K. & Nic Lughadha, E. M. (2015). Green Plants in the Red: A Baseline Global Assessment for the IUCN Sampled Red List Index for Plants. *PLoS ONE* 10(8): e0135152.
- Bustamante, E., Búrquez, A., Scheinvar, E. & Eguiarte, L. E. (2016). Population Genetic Structure of a Widespread Bat-Pollinated Columnar Cactus. *PLoS ONE* 11(3): e0152329.
- Contreras-Negrete, G., Ruiz-Duran, M., Cabrera-Tolledo, D., Casas, A., Vargas, O. & Parra, F. (2015). Genetic diversity and structure of wild and managed populations of *Polaskia chende* (Cactaceae) in the Tehuacan-Cuicatlan Valley, Central Mexico: insights from SSR and allozyme markers. *Genet. Resour. Crop Evol.* 62: 85 – 101.
- Duarte, M., Guerrero, P. C., Carvallo, G. & Bustamante, R. O. (2014). Conservation network design for endemic cacti under taxonomic uncertainty. *Biol. Conserv.* 176: 236 – 242.
- Dzialuk, A., Chybicki, I., Gout, R., Maćzka, T., Fleischer, P., Konrad, H., Curtu, A. L., Sofletea, N. & Valadon, A. (2014). No reduction in genetic diversity of Swiss stone pine (*Pinus cembra* L.) in Tatra Mountains despite high fragmentation and small population size. *Conserv. Genet.* 15: 1433 – 1445.
- Earl, D. A. & von Holdt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Res.* 4: 359 – 361.
- Eggle, U., Muñoz Schick, M. & Leuenberger, B. E. (1995). Cactaceae of South America: The Ritter collections. *Englera* 16. Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin.
- Elith, J., Phillips, S. J., Hastie, T., Dudik, M., Chee, Y. E. & Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists. *Divers. Distrib.* 17: 43 – 57.
- Ellstrand, N. C. & Elam, D. R. (1993). Population genetic consequences of small population size: Implications for plant conservation. *Annual Rev. Ecol. Syst.* 24: 217 – 242.
- El Mousadik, A. & Petit, R. J. (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theor. Appl. Genet.* 92: 832 – 839.
- Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of cluster of individuals using the software STRUCTURE: a simulation study. *Molec. Ecol.* 14: 2611 – 2620.
- Excoffier, L. & Lischer, H. E. L. (2010). Arlequin suite v. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Res.* 10: 564 – 567.
- Faundez, L., Walter, H. E., Guerrero, P. C. & Saldivia, P. (2013). *Copiapoa cinerea*. The IUCN Red List of Threatened Species 2013, e.T152039A590079.
- Fehlberg, S. D., Allen, J. M. & Church, K. (2013). A novel method of genomic DNA extraction for Cactaceae. *Appl. Plant Sci.* 1: 1200013.
- Figueredo, C. J., Nassar, J. M., Garcia-Rivas, A. E. & Gonzalea-Carcacia, J. A. (2010). Population genetic diversity and structure of *Pilosocereus tilianus* (Cactaceae, Cereaceae), a columnar cactus endemic to the Venezuelan Andes. *J. Arid Environ.* 74: 1392 – 1398.
- Frankham, R., Ballou, J. D. & Biscoe, D. A. (2010). Introduction to conservation genetics. Cambridge University Press, Cambridge.
- Funk, W. C., McKay, J. C., Hohenlohe, P. A. & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends Ecol. Evol.* 9: 489 – 496.
- Gaston, K. J. & Fuller, R. A. (2009). The sizes of species' geographic ranges. *J. Appl. Ecol.* 46, 1 – 9.
- Gayo, E. M., Latorre, C., Santoro, C. M., Maldonado, A. & De Pol-Holz, R. (2012). Hydroclimate variability in the low-elevation Atacama Desert over the last 2500 yr. *Clim. Past* 8: 287 – 306.
- Goetsch, B., Hilton-Taylor, C., Cruz-Piñón, G., Duffy, J. P., Frances, A., Hernández, H. M., Inger, R., Pollock, C., Schipper, J., Superina, M., Taylor, N. P., Tognelli, M., Abba, A. M., Arias, S., Arreola-Nava, H. J., Baker, M. A., Bárcenas, R. T., Barrios, D., Braun, P., Butterworth, C. A., Búrquez, A., Caceres, F., Chazaro-Basañez, M., Corral-Díaz, R., del Valle Perea, M., Demaió, P. H., Duarte de Barros, W. A., Durán, R., Faúndez Yancas, L., Felger, R. S., Fitz-Maurice, B., Fitz-Maurice, W. A., Gann, G., Gómez-Hinostrosa, C., Gonzales-Torres, L. R., Griffith, M. P., Guerrero, P. C., Hammel, B., Heil, K. D., Guadalupe Hernández-Oria, J., Hoffmann, M., Ishiki Ishihara, M., Kiesling, R., Larocca, J., León-de la Luz, J. L., Loaiza, S. C. R., Lowry, M., Machado, M. C., Majure, L. C., Guadalupe Martínez Ávalos, J., Martorell, C., Maschinski, J., Méndez, E., Mittermeier, R. A., Nassar, J. M., Negrón-Ortiz, V., Oakley, L. J., Ortega-Baes, P., Pin Ferreira, A. B., Pinkava, D. J., Porter, J. M., Puente-Martínez, R., Roque Gamarra, J., Saldivia Pérez, P., Sánchez Martínez, E., Smith, M., del Sotomayor, M. C. J. M., Stuart, S. N., Tapia Muñoz, J. L., Terrazas, T., Terry, M., Trevisson, M., Valverde, T., Van Devender, T. R., Véliz-Pérez, M. E., Walter, H. E., Wyatt, S. A., Zappi, D., Zavala-Hurtado, J. A. & Gaston, K. J. (2015). A high proportion of cactus species threatened with extinction. *Nature Plants* 1: 15142.
- Goudet, J. (1995). FSTAT (v. 1.2): a computer program to calculate F-statistics. *J. Hered.* 86: 485 – 486.

- (2002). FSTAT: a program to estimate and test gene diversities and fixation indices (v. 2.9.3.2). <http://www2.unil.ch/popgen/softwares/fstat.htm/> (accessed 29.06.2015).
- Guerrero, P. C., León-Lobos, P. & Squeo, F. (2010). Ficha de antecedentes de especie: *Copiapoa krainziana*. http://www.mma.gob.cl/clasificacionespecies/fichas6proceso/fichas2010/Copiapoakrainziana_P06R2_RCE.pdf (accessed 05.05.2015).
- , Durán, A. P. & Walter, H. E. (2011a). Latitudinal and altitudinal patterns of the endemic cacti from the Atacama Desert to mediterranean Chile. *J. Arid. Environ.* 75: 991 – 997.
- , Arroyo, M. T. K., Bustamante, R. O., Hagemann, T. & Walter, H. E. (2011b). Phylogenetics and predictive distribution modeling provide insights into infrageneric relationships and the evolution of the *Eriosyce* subgen. *Neoporteria* (Cactaceae). *Pl. Syst. Evol.* 297: 113 – 128.
- , León-Lobos, P. & Squeo, F. (2012). Ficha de antecedentes de especie: *Copiapoa cinerea*. www.mma.gob.cl/clasificacionespecies/ficha5proceso/fichas_actualizadas/Copiapoa_cinerea_P05R5-9_RCE.doc (accessed 05.05.2015).
- Guo, S. W. & Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48: 361 – 372.
- Hamrick, J. L., Nason, J., Fleming, T. H. & Nassar, J. M. (2002). Genetic diversity in columnar cacti. In: T. H. Fleming & A. Valiente-Banuet (eds), *Evolution, ecology and conservation of columnar cacti and their mutualists*, pp. 122 – 133. University of Arizona Press, Arizona.
- Hardesty, B. D., Hughes, S. L., Rodriguez, V. M. & Hawkins, J. A. (2008). Characterization of microsatellite loci for the endangered cactus *Echinocactus grusonii*, and their cross-species utilization. *Molec. Ecol. Res.* 8: 164 – 167.
- Hartl, D. L. & Clark, A. G. (1997). *Principles of Population Genetics*, 3rd edn. Sinauer Associates, Inc, Sunderland, MA.
- Hernández-Hernández, T., Brown, J. W., Schlumpberger, B. O., Eguiarte, L. E. & Magallón, S. (2014). Beyond aridification: Multiple explanations for the elevated diversification of cacti in the New World Succulent Biome. *New Phytol.* 202: 1382 – 1397.
- Hoffmann, A. E. & Flores, A. R. (1989). The conservation status of Chilean succulent plants: a preliminary assessment. In: I. Benoit (ed.), *Red List of Chilean Terrestrial Flora*, pp. 107 – 121. Corporación Nacional Forestal, Santiago.
- & Walter, H. E. (2004). *Cactáceas en la flora silvestre de Chile*, 2nd edn. Fundación Claudio Gay, Santiago.
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25: 1965 – 1978.
- Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N. & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecol. Lett.* 11: 609 – 623.
- Hunt, D. (2002). *Copiapoa cinerea* subsp. *columna-alba*. *Cact. Syst. Init.* 13: 13.
- , Taylor, N. & Charles, G. [eds] (2006). *The New Cactus Lexicon: Descriptions and illustrations of the cactus family* compiled and edited by members of the International Cactaceae Systematics Group. Vols 1, 2. DH Books, Milborne Port.
- Isaac, N. J., Mallet, J. & Mace, G. M. (2004). Taxonomic inflation: its influence on macroecology and conservation. *Trends Ecol. Evol.* 19: 464 – 469.
- IUCN (2012). *IUCN red list categories and criteria*: version 3.1, Vol. 4, 2nd edn. IUCN, Cambridge & Gland.
- (2015). *The IUCN Red List of Threatened Species*. Version 2015-4. <http://www.iucnredlist.org/> (accessed 21.11.2015).
- Jakobsson, M. & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801 – 1806.
- Jombart, T. & Ahmed, I. (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* 27: 3070 – 3071.
- Jump, A. S., Marchant, R. & Penuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends Plant Sci.* 14: 51 – 58.
- Kearse, M., Moir, R., Wilson, A., Stones-Haves, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647 – 1649.
- Kramer, A. T. & Havens, K. (2009). Plant conservation genetics in a changing world. *Trends Plant Sci.* 14: 599 – 607.
- Larridon, I., Shaw, K., Cisternas, M. A., Paizanni Guillén, A., Sharrock, S., Oldfield, S., Goetghebeur, P. & Samain, M.-S. (2014). Is there a future for the Cactaceae genera *Copiapoa*, *Eriosyce* and *Eulychnia*? A status report of a prickly situation. *Biodivers. Conserv.* 23: 1249 – 1287.
- , Walter, H. E., Guerrero, P. C., Duarte, M., Cisternas, M. A., Peña-Hernández, C., Bauters, K., Asselman, P., Goetghebeur, P. & Samain, M.-S. (2015). An integrative approach to understanding the evolution and diversity of *Copiapoa* (Cactaceae), a threatened endemic genus from Chile's Atacama Desert. *Amer. J. Bot.* 102: 1506 – 1520.
- Levy, F. & Neal, C. (1999). Spatial and temporal genetic structure in chloroplast and allozyme

- markers in *Phacelia dubia* implicate genetic drift. *Heredity* 82: 422 – 431.
- Lewontin, R. C. & Kojima, K. (1960). The evolutionary dynamics of complex polymorphisms. *Evolution* 14: 450 – 472.
- Luebert, F. & Pliscoff, P. (2006). *Sinopsis bioclimática y vegetacional de Chile*. Editorial Universitaria, Santiago.
- Majure, L. C., Puente, R., Griffith, M. P., Judd, W. S., Soltis, P. S. & Soltis, D. S. (2012). Phylogeny of *Opuntia* s.s. (Cactaceae): clade delineation, geographic origins, and reticulate evolution. *Amer. J. Bot.* 99: 847 – 864.
- Markert, J. A., Champlin, D. M., Gutjahr-Gobell, R., Grear, J. S., Kuhn, A., McGreevy, T. J., Roth, A., Bagley, M. J. & Nacci, D. E. (2010). Population genetic diversity and fitness in multiple environments. *BMC Evol. Biol.* 10: 205.
- Michalakis, Y. & Excoffier, L. (1996). A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 142: 1061 – 1064.
- Nassar, J. M., Hamrick, J. L. & Fleming, T. H. (2003). Population genetic structure of Venezuelan chiropterophilous columnar cacti (Cactaceae). *Amer. J. Bot.* 90: 1628 – 1637.
- Ohmura, A., Dutton, E. G., Forgan, B., Fröhlich, C., Gilgen, H., Hegner, H., Heimo, A., König-Langlo, G., McArthur, B., Müller, G., Philipona, R., Pinker, R., Whitlock, C. H., Dehne, K. & Wild, M. (1998). Baseline Surface Radiation Network (BSRN)/WCRP): New precision radiometry for climate research. *Bull. Amer. Meteorol. Soc.* 79: 2115 – 2136.
- Ossa, C. G., Larridon, I., Peralta, G., Asselman, P. & Pérez, F. (2016). Development of microsatellite markers using Next-Generation Sequencing for the columnar cactus *Echinopsis chiloensis* (Cactaceae). *Molec. Biol. Rep.* 43 (12): 1315 – 1320.
- Ouborg, N. J., Vergeer, P. & Mix, C. (2006). The rough edges of the conservation genetics paradigm for plants. *J. Ecol.* 94: 1233 – 1248.
- Philippi, R. A. (1860). *Florula Atacamensis seu Enumeratio Plantarum in itinere per desertum Atacamense observatarum*. Eduard Anton, Halle.
- Phillips, S. J., Anderson, R. P. & Schapire, R. E. (2006). Maximum entropy modelling of species geographic distributions. *Ecol. Model.* 190: 231 – 259.
- Pliscoff, P., Luebert, F., Hilger, H. H. & Guisan, A. (2014). Effects of alternative sets of climatic predictors on species distribution models and associated estimates of extinction risk: A test with plants in an arid environment. *Ecol. Model.* 288: 166 – 177.
- Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype Data. *Genetics* 155: 945 – 959.
- Raymond, M. & Rousset, F. (1995). GENEPOP (v. 1.2.): population genetics software for exact tests and ecumenicism. *J. Hered.* 86: 248 – 249.
- R Core Team (2015). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org> (accessed 15.09.2015).
- Reed, D. H. & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conserv. Biol.* 17: 230 – 237.
- Ritter, F. (1959). *Copiapoa columna-alba*. *Cactus (Paris)* 14 (65): 199 – 200.
- (1963). Diagnosen von neuen kakteen. *Taxon* 12 (1): 28 – 34.
- (1980). *Kakteen in Südamerika*, Band 3. Friedrich Ritter Selbstverlag, Spangenberg.
- Rosenberg, N. A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molec. Ecol. Notes* 4: 137 – 138.
- Rossetto, M. & Rymer, P. (2013). Applications of molecular markers in plant conservation. In: R. J. Henry (ed.), *Molecular Markers in Plants*, pp. 81 – 98. Wiley-Blackwell Publishing, Singapore.
- Rousset, F. (2008). GENEPOP'007: a complete reimplementation of the GENEPOP software for Windows and Linux. *Molec. Ecol. Res.* 8: 103 – 106.
- Saldivia, P., Guerrero, P. C., Faundez, L. & Walter, H. E. (2013). *Copiapoa krainziana*. *The IUCN Red List of Threatened Species* 2013, e.T152458A639215.
- Schulz, R. (2006). *Copiapoa*. Schulz Publishing, Teesdale.
- Schulz, R., & Kapitany, A. (1996). *Copiapoa in their environment: Chañaral to El Cobre*. Southbank Book, Teesdale.
- Slaba, R. (1997). *Copiapoa cinerea* subsp. *krainziana*. *Kaktusy (Brno)* 33 (special): 3.
- Slatkin, M. (1994). Linkage disequilibrium in growing and stable populations. *Genetics* 137, 331 – 336.
- (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457 – 462.
- & Excoffier, L. (1996). Testing for linkage disequilibrium in genotypic data using the Expectation-Maximization algorithm. *Heredity* 76: 377 – 383.
- Smith, P. T. (2013). *Genetic partitioning within the metapopulation of endangered Bakersfield cactus (Opuntia basilaris var. treleasei): implications for translocation efforts*. California State University-Bakersfield, Bakersfield, CA.
- Solorzano, S., Cuevas-Alducin, P. D., Garcia-Gomez, V. & Davila, P. (2014). Genetic diversity and conservation of *Mammillaria huitzilopochtli* and *M. supertexta*, two threatened species endemic of the semiarid region of central Mexico. *Rev. Mex. Biodivers.* 85: 565 – 575.
- Syfert, M. M., Joppa, L., Smith, M. J., Coomes, D. A., Bachman, S. P. & Brummitt, N. A. (2014). Using species distribution models to inform IUCN Red List assessments. *Biol. Conserv.* 177: 174 – 184.
- Taylor, N. P. (1997). *Copiapoa cinerea* subsp. *haseltoniana*. *Cact. Syst. Init.* 3: 8.
- Terry, M., Pepper, A. E. & Manhart, J. R. (2006). Development and characterization of microsatellite

- loci in endangered *Astrophytum asterias* (Cactaceae). *Molec. Ecol. Notes* 6: 865 – 866.
- , ——, Strong, A. W., Tarin, D. M., Price, D. M. & Manhart, J. R. (2012). Genetic structure of a population of the endangered star cactus (*Astrophytum asterias*) in southern Texas. *Southw. Naturalist* 57: 182 – 188.
- Tinoco, A., Casas, A., Luna R. & Oyama, K. (2005). Population genetic of wild and silvicultural managed populations of *Escontria chiotilla* in the Tehuacan Valley, central Mexico. *Genet. Resour. Crop. Evol.* 52: 525 – 538.
- UNEP (2015). *The Species+ Website*. Nairobi, Kenya. Compiled by UNEP-WCMC, Cambridge. <http://www.speciesplus.net/> (accessed 07.12.2015).
- Walter, H. E. (2011). Protecting cacti: a pending and urgent task in Chile's biodiversity conservation. In: E. Figueroa B. (ed.), *Biodiversity Conservation in the Americas: Lessons and Policy Recommendations*, pp. 47 – 80. Ocho Libros Editores Ltda, Santiago.
- Warren, D., Glor, R. E. & Turelli, M. (2008). Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution* 62: 2868 – 2883.
- Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358 – 1370.
- Wright, S. (1978). *Evolution and the Genetics of Population, Variability Within and Among Natural Populations*. The University of Chicago Press, Chicago.
- Zomer, R. J., Bossio, D. A., Trabucco, A., Yuanjie, L., Gupta, D. C. & Singh, V. P. (2007). *Trees and Water: Smallholder agroforestry on irrigated lands in northern India*. IWMI Research Report 122, International Water Management Institute, Colombo.
- , Trabucco, A., Bossio, D. A., van Straaten, O. & Verchot, L. V. (2008). Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agric. Ecosyst. Environ.* 126: 67 – 80.